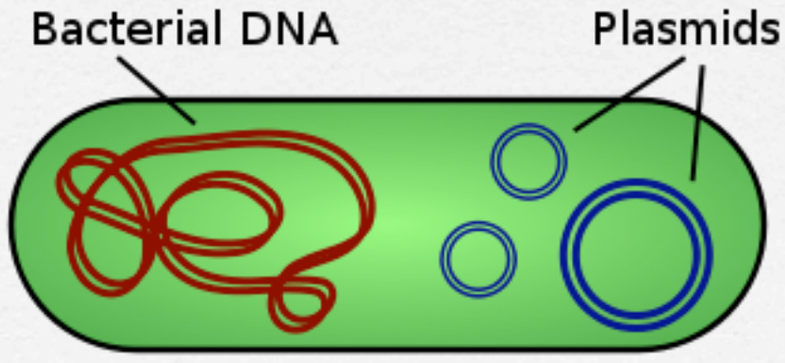
Synthetic Biology for iGEM

Plasmids and DNA Digestion

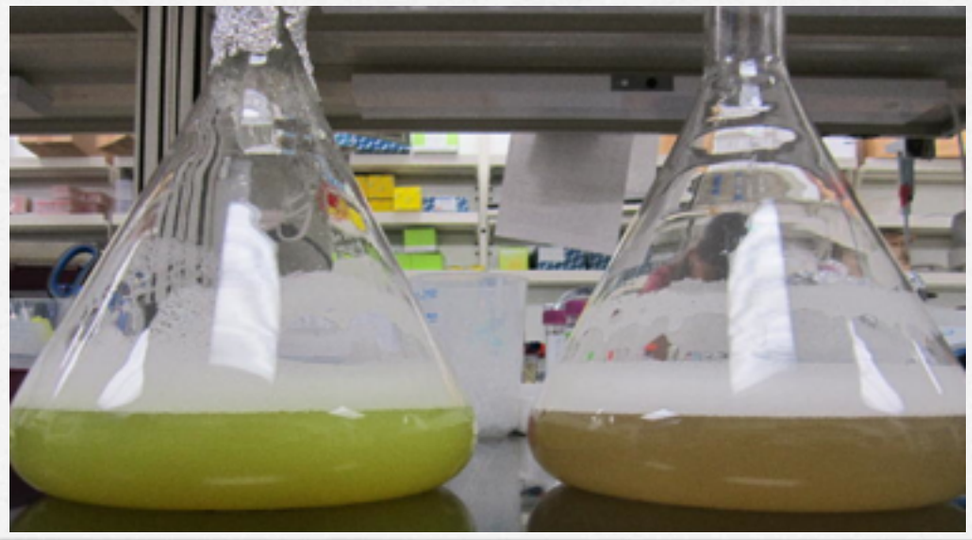
Plasmids

- Plasmids are small DNA molecules that are separate from chromosomal DNA
- They are most commonly found as double stranded, circular DNA
- Typical plasmids range from 1-100 kbp (1,000 to 100,000 base pairs)



Plasmids in Synthetic Biology

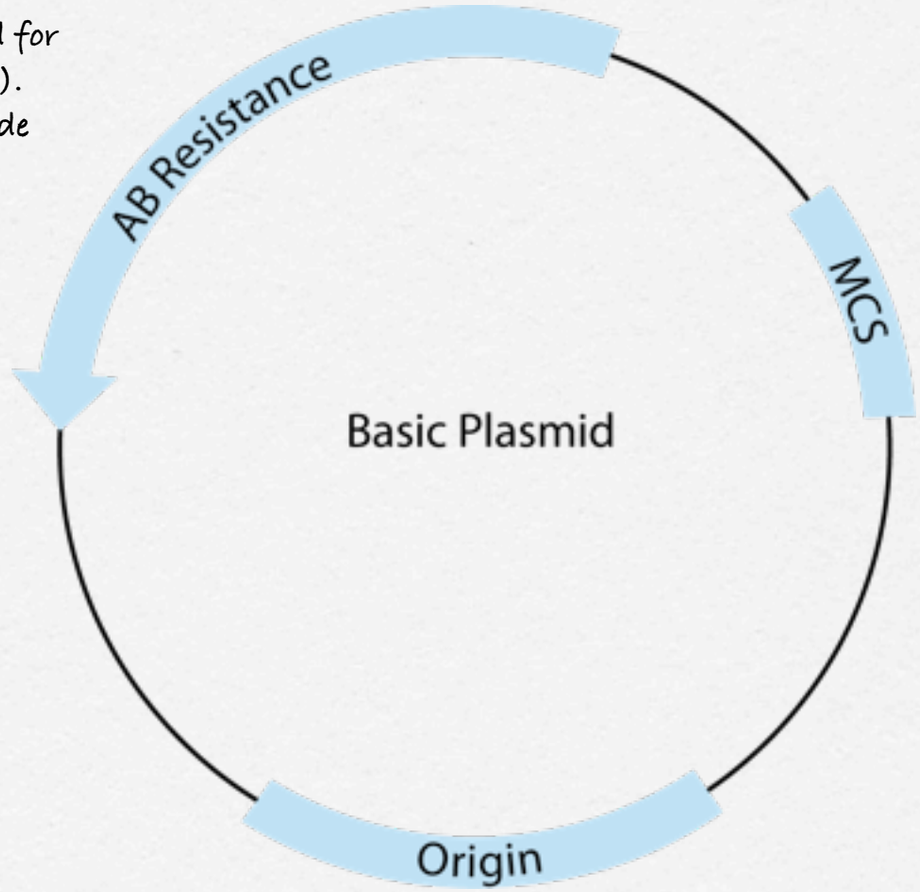
- Plasmids are a way of introducing DNA into bacteria (like E. coli)
- Bacteria will express plasmid DNA under the right conditions
- By introducing DNA coding for proteins into plasmids, we can get E. coli to produce protein in large quantities



Two bacterial cultures. The culture on the left is expressing GFP (from plasmid DNA). The one on the right is not.

Plasmid Composition

Antibiotic Resistance. Used for selection. (See later slides). Example antibiotics include ampicillin (Amp) and kanamycin (kan)

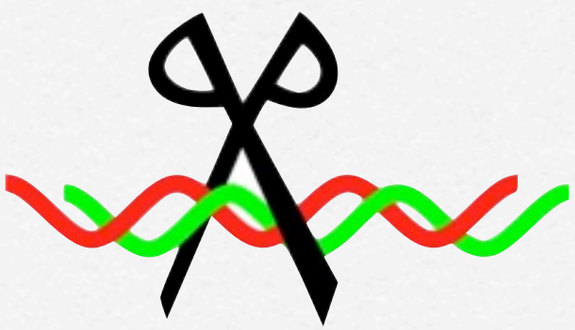


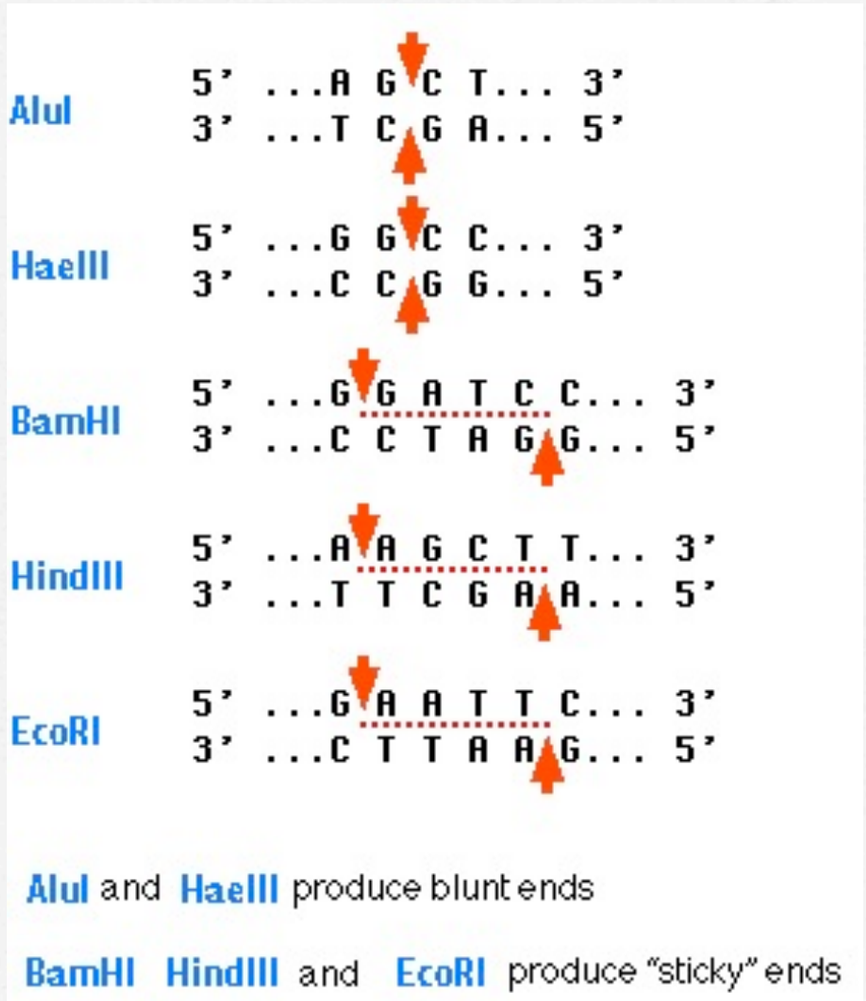
Multiple Cloning Site. This area contains sites for restriction enzymes to cut and paste in different DNA.

Origin of Replication. This is important so that when bacteria replicate, the plasmid is also replicated.

Restriction Enzymes

- The "scissors" of synthetic biology
- Restriction enzymes recognize specific DNA sequences and cut them
- Restriction enzymes are a bacterial defense from foreign DNA (like viruses)



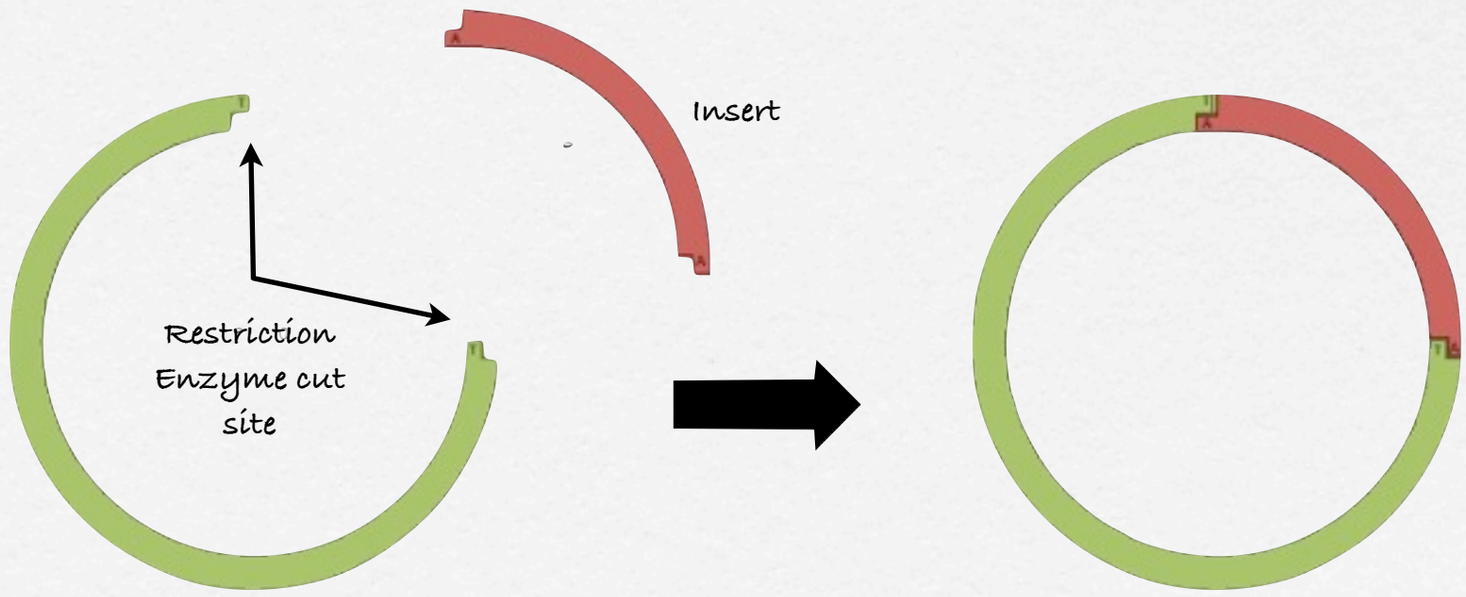


Blunt Ends vs. Sticky Ends

- Restriction Enzymes that cut with blunt ends produce two double stranded DNA products
- Restriction enzymes that cut with sticky ends leave single stranded DNA overhangs in their products

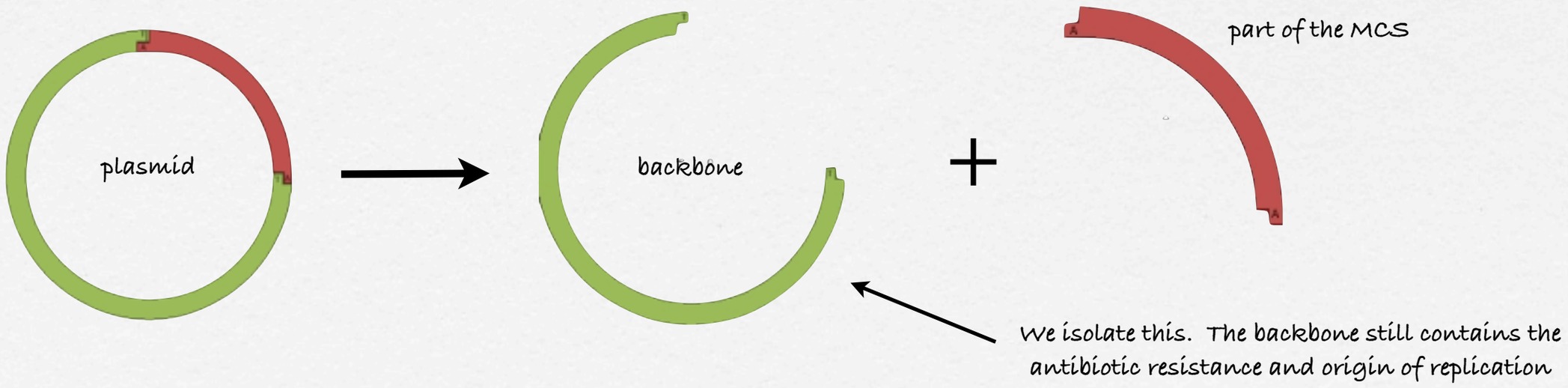
The Multiple Cloning Site

- Contains many sites for restriction enzymes
- Scientists use the restriction enzymes to cut out sections of the plasmid, and add in their own genes of interest, termed inserts
- The insertion reaction is called ligation



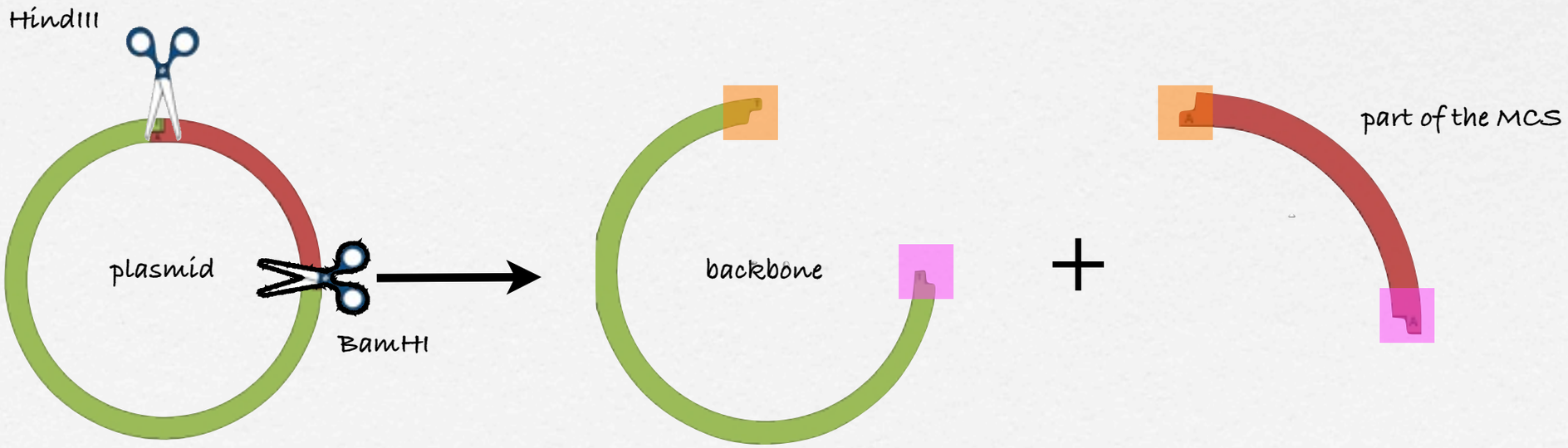
Experimental Methods: Plasmid Digestion

- Overview of the experiment: Digest an MCS of a plasmid and isolate the plasmid backbone
- Purpose: Collect a strand of plasmid DNA that can be linked to our gene of interest



Plasmid Digestion: Restriction Enzymes

- Typical digestions cut in with two different restriction enzymes
- Both cuts result in sticky ends
- Because the sticky ends are different, the plasmid will not join with itself



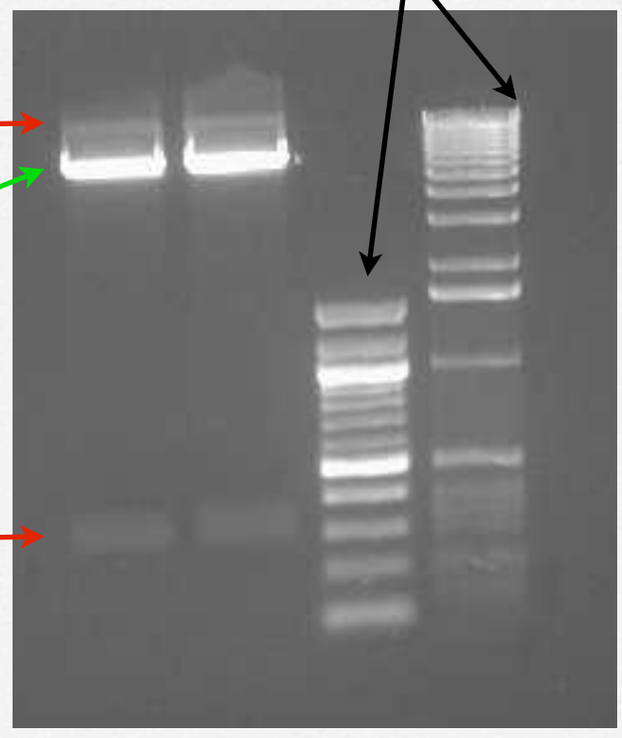
Plasmid Digestion: Extracting the backbone

DNA ladders. DNA ladders contain DNA of known size, and are run along with experiment lanes as a standard for measurement of DNA size.

Whole plasmid. We don't want this.

Backbone only. This piece must be carefully cut from the gel.

Insert only. We don't want this.



- Plasmid backbones are separated and extracted by DNA gel (agarose) electrophoresis
- The gel separates the DNA based on the size (number of base pairs)

Agarose Gels

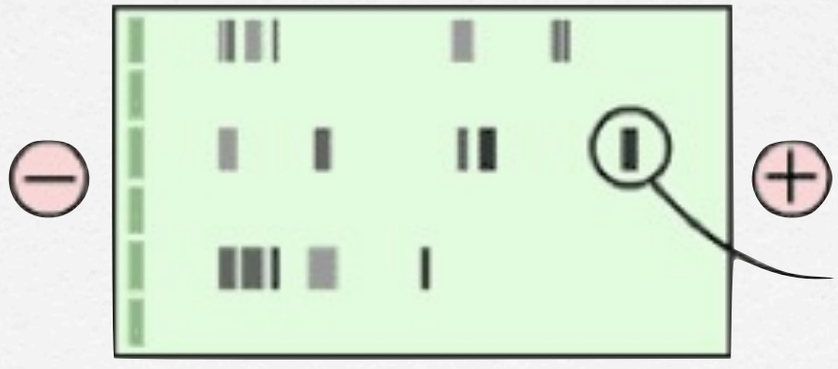
- Agarose is a polysaccharide (made of many sugars linked together)
- When agarose is linked together it forms a porous gel
- This gel is porous enough to allow molecules like DNA to move through it

DNA Electrophoresis

- Electrophoresis is the process of moving a biomaterial (usually DNA or protein) through a gel phase material using electric potential

- DNA is negatively charged (phosphate group), and so moves from the negative electrode (cathode) to the positive electrode (anode)

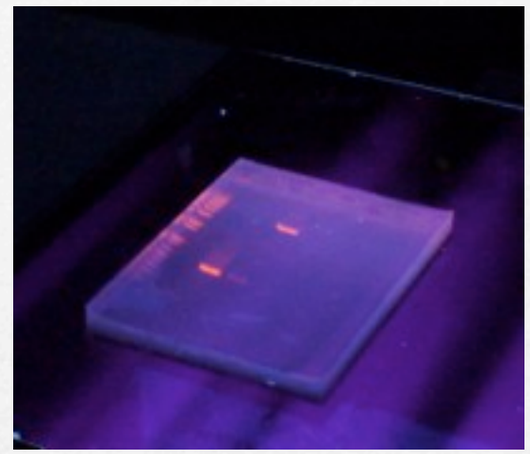
- Larger DNA molecules move more slowly through the gel because of collisions with the gel matrix

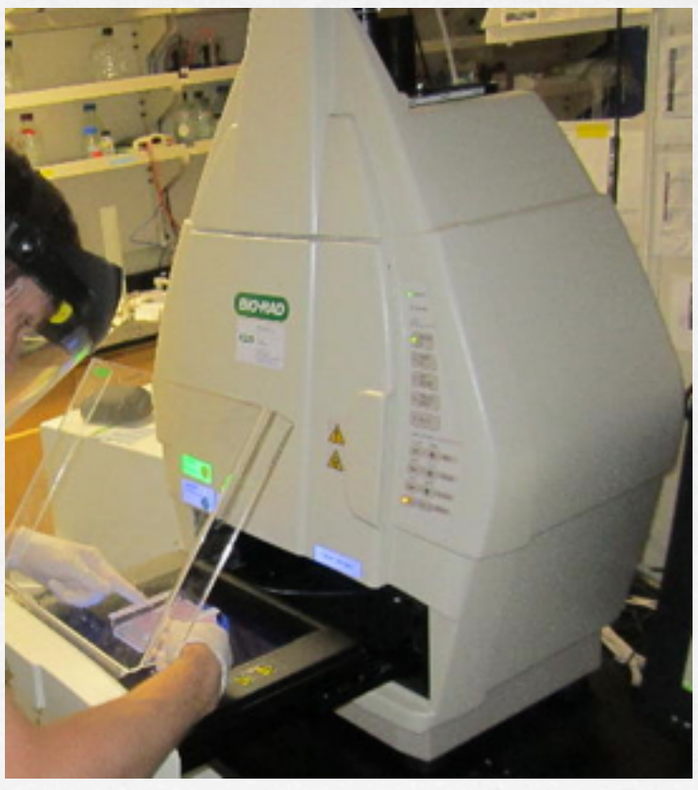


DNA Extraction

- We visualize the DNA by using DNA-binding molecules, such as Ethidium Bromide (EtBr)
- Ethidium bromide allows the DNA to be seen in UV light
- Extract the DNA bands using a razor blade

DNA gel highlighted under UV light. Ethidium Bromide makes the DNA appear orange.

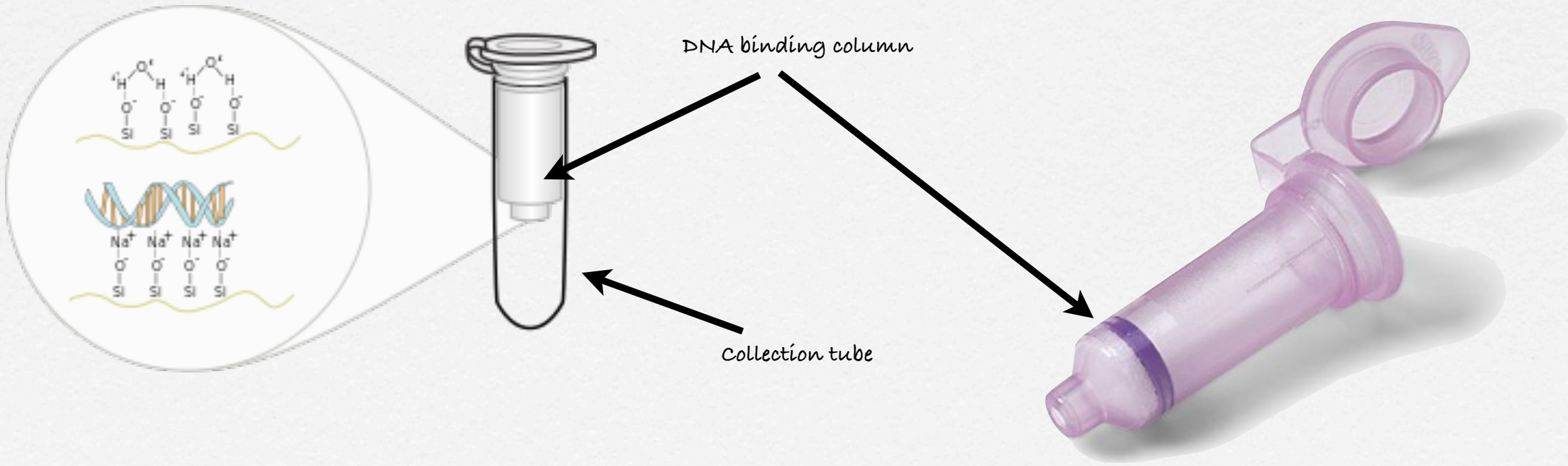




DNA extraction using a razor blade

DNA Purification

- DNA is purified through column purification
- Many companies sell kits for this column purification

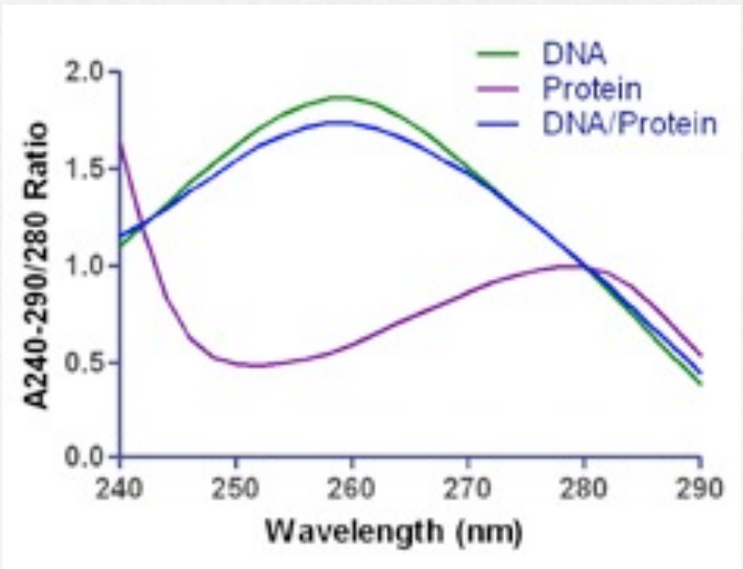


Experimental Methods: DNA Purification

- Gel Digestion: Soak excised gel in warm dissolving fluid to bring everything into solution
- DNA binding: Load the solution onto the column and spin down with a centrifuge. DNA will get trapped on the column
- Wash phases: Load wash buffer onto the column and spin down. The wash buffer gets rid of materials that have bound to the column nonspecifically. Usually there are several wash phases
- Elution: A special buffer (sometimes just water) is sent through the column which promotes the release of the DNA. A fresh collection tube is used for this step to prevent contamination.

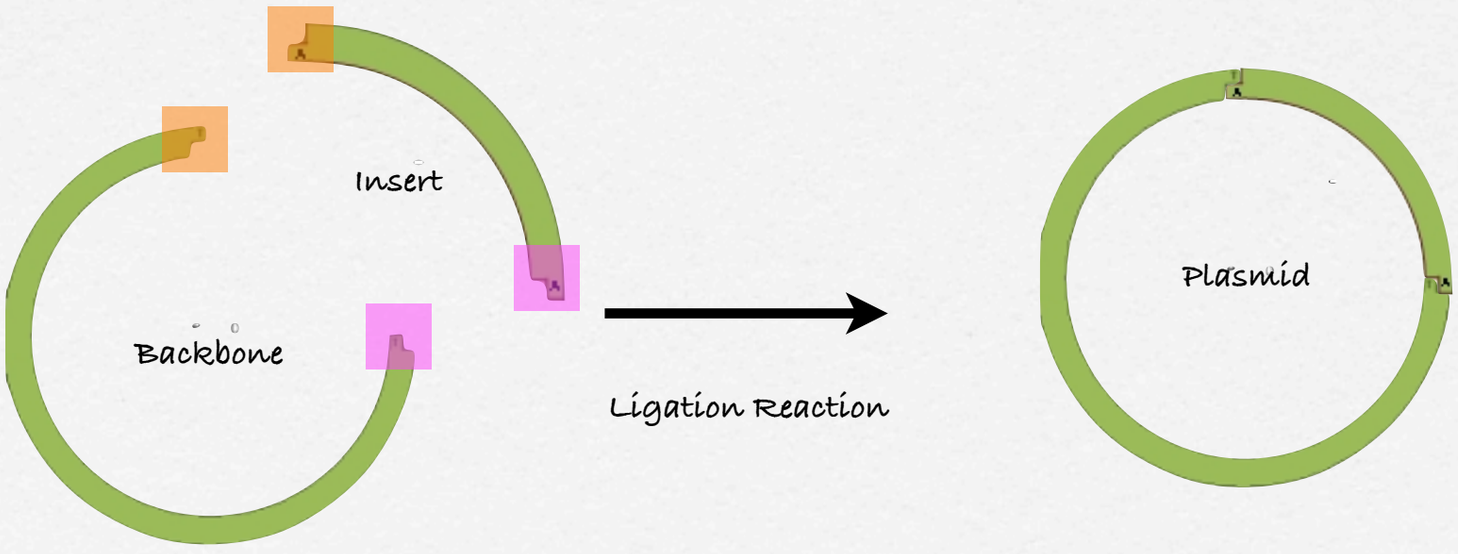
Measuring DNA Concentration

- DNA concentration is measured using a nanodrop or spectrophotometer
- DNA has strong absorbance at 260 nm, and low absorbance at 280 nm
- The ratio of 260/280 tells the DNA concentration



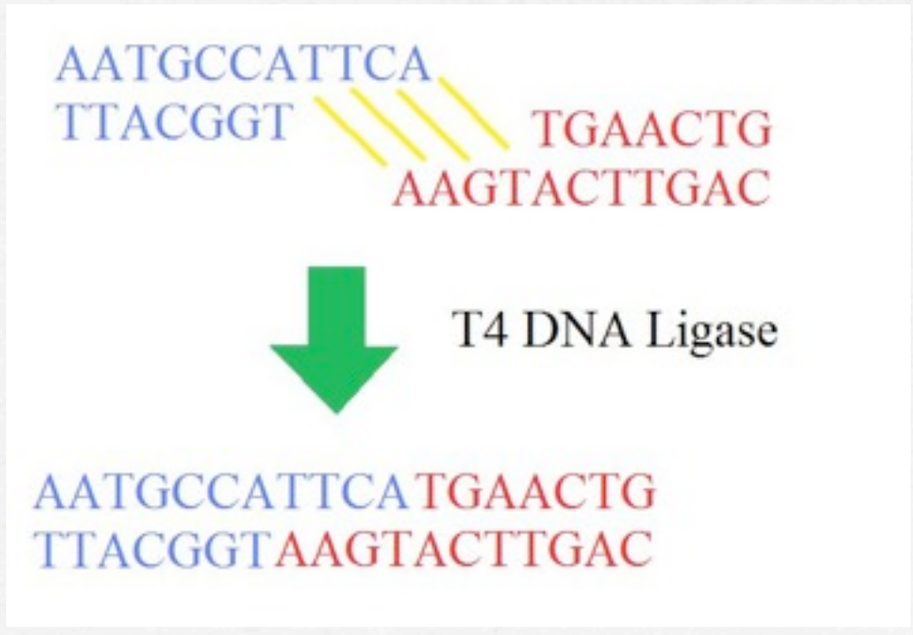
Ligation

- After the plasmid backbone has been isolated, we now want to add in the DNA for the protein of interest, called the insert
- This is called a ligation reaction, and is carried out by the enzyme DNA ligase
- Insert has been digested with same two restriction enzymes as the backbone to create complementary overlaps



Experimental Methods: Ligation

- DNA ligase is mixed with plasmid and insert for several hours (20 C) or overnight (4 C)
- To test the success of the reaction, plasmid is transformed into bacteria for cloning (see slides on transformation/cloning)



Review

- Plasmids are circular pieces of DNA that can be manipulated in vitro and introduced into bacteria
- Plasmid manipulation utilizes cutting (restriction enzymes) and pasting (DNA ligase) techniques
- Agarose gels are used to separate and visualize DNA

Important Terminology

Vector: DNA (or RNA) used to artificially carry foreign material into another cell.
Plasmid: Circular piece of double stranded DNA used as a vector for bacterial cells. A plasmid is a vector but not all vectors are plasmids.
Multiple Cloning Site (MCS): A region of the plasmid containing many restriction enzyme sites. The MCS is used to insert target genes into the plasmid.
Origin of Replication: Sequence denoting a starting point for DNA replication There is at least one replication of origin in genomic DNA, and plasmids must have one in order to be replicated during cell division.
Restriction Enzyme: Special type of enzyme which "cuts" DNA, breaking the double helix. Restriction enzymes recognize target DNA sequences (usually palindromic).
Agarose: A type of carbohydrate (sugar). Agarose is frequently polymerized into long chains to form gels that are suitable for DNA electrophoresis.
Electrophoresis: A method of separation, typically for DNA or proteins, which uses the charge of the molecules to drive separation. Smaller molecules move faster through the gel matrix than large ones.
Ethidium Bromide: A compound frequently used to visualize DNA. Ethidium Bromide binds to DNA and allows it to be seen under UV light. Ethidium bromide is highly carcinogenic.
Column Purification: A method of purification used in many applications. In general, column purification relies on the target binding to the column while other molecules wash through. The final phase of column purification is elution, where the target molecule is unbound from the column with a special buffer and collected.
Nanodrop: A machine used to measure concentrations. Nanodrops measure absorbance, and can calculate concentration based on absorbance spectrum and laws, such as Beer's Law.
DNA Ligase: An enzyme whose function is to fuse together two pieces of DNA. DNA ligase repairs broken bonds in the DNA backbone and does not add nucleotides like DNA polymerase.